PROTOCOL FOR CHEMOTHERAPY OF SUBCUTANEOUSLY IMPLANTED H460 LARGE CELL LUNG CARCINOMA XENOGRAFT

MODEL: (3CLH2) Subcutaneously Implanted H460 Large Cell Lung Carcinoma Xenograft

Origin of Tumor Line: (No details).

Summary of Test Procedures: A tumor fragment is implanted s.c. in the axillary region of athymic random bred (NCr-nu) mice. Test agent treatment starts when the tumors reach a weight range of 200-600 mg. The parameter is change in mean tumor weight. Results are expressed as a percentage of the control tumor weight.

ANIMALS: (refer to Protocol 8)

Propagation: Athymic random bred (NCr-nu) mice.

Testing: Athymic random bred (NCr-nu) mice.

Weight: Mice should be within a 5 gm weight range, with a minimum weight of 18 gm for males and 17 gm for females.

Sex: One sex is used for all test and control animals in one experiment.

Source: One source, if feasible, for all animals in one experiment. Exceptions to be noted in comments.

EXPERIMENT SIZE: (refer to Protocol 9)

General Testing: Six earmarked animals per test group.

Control Group: A minimum of 20 control animals must be used; otherwise, the number of control animals varies according to the number of test groups.

TUMOR TRANSFER: (refer to Protocols 2, 5, and 6).

PROPAGATION

Fragment: Prepare a 30 mg (acceptable range 20-40 mg) fragment from 500-800 mg s.c. donor tumor without surface ulceration.

Time: When donor tumor reaches 500-800 mg (approximately Day 10-14 after implant).

Site: Implant 30 mg fragment s.c. into axillary region with puncture in inguinal region.

TESTING:

Fragment: Prepare a 30 mg (acceptable range 20-40 mg) fragment from 500-800 mg s.c. donor tumor without surface ulceration.

Time: When donor tumor reaches 500-800 mg (approximately Day 10-14 after implant).

Site: Implant 30 mg fragment s.c. into axillary region with puncture in inguinal region.

TESTING SCHEDULE: (refer to Protocols 3 and 4).

Day 0: Implant tumor. Run bacterial cultures (refer to Protocol 7).

Day 1: Check cultures. Discard experiment if contaminated.

Day 2: Recheck cultures. Discontinue if contaminated and report accordingly.

Staging Day (Initial Treatment Day): Select mice with tumors weighing no less than 200 mg and no more than 600 mg. Prepare materials. Randomize animals and treat by individual body weight. Inject test agent on Staging Day and continue as indicated by the experimental design. Record total group body weights (Weigh Day 1). Record deaths daily.

Measurement Days: Body weights and tumor measurements are recorded on Initial Treatment Day (Staging Day). Additionally, weigh animals at the end of the treatment period and/or at any time specified by the experimental design. Measure tumors twice weekly.

Final Evaluation Day: Variable - dependent upon the experimental design; that measurement day which yields the optimum (best) T/C% is designated Final Evaluation Day. End and evaluate experiment.

QUALITY CONTROL: (refer to Protocol 7)

Not established.

EVALUATION: (refer to Protocol 11).

The parameter measured is mean tumor weight change (delta) based on length and width measurements in millimeters. Tumors are considered eligible for evaluation in the period 7 to 35 days post treatment for single injection treatment and 35 days post treatment for all other regimens. Measurement days should be selected such that the entire evaluation eligibility period is examined (i.e., post initial treatment days 13, 17, and 21 as an example). T/C% is calculated for only those groups where the survivors on the final possible evaluation day are greater than 65%. An excessive animal body weight change difference (test minus control) may also be used in evaluating toxicity.

Parameter: Calculations are based on group weights. Animals are earmarked only to permit total elimination of data for any animal designated as a no-take, any animal that escapes, or any animal that for any reason is deemed by the screener to be unacceptable for inclusion in the calculations.

Mean tumor weights are calculated for each measurement day within the eligibility period as each day is potentially a Final Evaluation Day. For each such day, change (delta) in mean tumor weight is calculated for both the test and control, as follows:

1. Calculate tumor weights (mgs) from tumor dimensions (mm x mm) following the formula for the volume of a prolate ellipsoid:

$$\frac{L \times W^2}{2}$$ Where L is the longer of the two measurements.

2. Calculate the change (delta) in mean tumor weight for test (T) and control (C) groups of mice:

Change (delta) in Mean Tumor Weight (ΔWt) = Mean Tumor Weight $_{FINAL}$ - Mean Tumor Weight $_{INITIAL}$.

3. Calculate T/C% for all test groups with >65% survivors on Final Evaluation Day:

$$T/C\% = \frac{\Delta WtT}{\Delta WtC} \times 100$$ -- if ΔWtT positive.

$$T/C\% = \frac{\Delta WtT}{\text{Test Mean Tumor Weight}_{INITIAL}} \times 100$$ -- if ΔWtT Negative.

Determine optimum T/C% and the day of its occurrence to establish the final T/C% evaluation and Final Evaluation Day.

CRITERIA FOR ACTIVITY:

An initial T/C $\leq$20% is considered necessary to demonstrate moderate activity.

A reproducible T/C $\leq$10% is considered significant activity.

REPORTING OF DATA:

On the final day of testing, prepare final control and test reports.

Assign a Test Status Code (TSC) of 33 to any test group the screener considers to be invalid for any reason.

A comment must be provided stating the reason for a TSC of 33, when a nonstandard dose is administered (whether due to a solubility problem or special request), and for poor suspensions.

EVALUATION FOR CELL KILL:

1. First, compute a T-C value using two points on the growth curve as follows:

EXAMPLE:

TREATED GROUP	
a)	Point 1: Day of appearance to 1000 mg = 20
b)	Point 2: Day of appearance to 1500 mg = 22

CONTROL GROUP	
c)	Point 1: Day of appearance to 1000 mg = 10
d)	Point 2: Day of appearance to 1500 mg = 12

T-C	
(a-c)	20-10 = 10 day T-C to 1000 mg
(b-d)	22-12 = 10 day T-C to 1500 mg

Mean value = 20/2 = 10 day T-C

2. Compute net cell kill at the end of the treatment period using the following formula:

$$K_E = \frac{\log 2}{Td} [(T-C)-(n-1)I]$$

where:

K_E = Net cell kill at the end of the treatment period (Log_{10} units)

Td = Tumor volume doubling time (Days)

T-C = Difference in appearance of the treated and control tumors to the same size (Days).

n = The length of the treatment period (Days).

I = The interval between treatments (Days).